

Effect of Drinking Rate on the Retention of Water or Milk Following Exercise-Induced Dehydration

Sayer, Liam; Rodriguez-Sanchez, Nidia; Rodriguez-Giustiniani, Paola; Irwin, Christopher; McCartney, Danielle; Cox, Gregory R; Galloway, Stuart D R; Desbrow, Ben

Published in:
International Journal of Sport Nutrition and Exercise Metabolism

DOI:
[10.1123/ijsnem.2019-0176](https://doi.org/10.1123/ijsnem.2019-0176)

Licence:
Other

[Link to output in Bond University research repository.](#)

Recommended citation(APA):
Sayer, L., Rodriguez-Sanchez, N., Rodriguez-Giustiniani, P., Irwin, C., McCartney, D., Cox, G. R., Galloway, S. D. R., & Desbrow, B. (2020). Effect of Drinking Rate on the Retention of Water or Milk Following Exercise-Induced Dehydration. *International Journal of Sport Nutrition and Exercise Metabolism*, 30(2), 128-138.
<https://doi.org/10.1123/ijsnem.2019-0176>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

For more information, or if you believe that this document breaches copyright, please contact the Bond University research repository coordinator.

1 **Introduction**

2 Individuals typically do not consume enough fluid during exercise to counteract sweat losses, producing a
3 post-exercise state of body water deficit (i.e. dehydration) (Garth & Burke, 2013). As a result, individuals
4 are encouraged to drink fluid during recovery to reinstate total body water balance prior to recommencing
5 physical activity (Evans et al., 2017; Sawka et al., 2007). However, rapidly consuming large volumes of
6 hypotonic fluid has the potential to reduce plasma osmolality (POSM), resulting in increased urinary
7 output (i.e. “fluid induced diuresis”), potentially delaying a return to euhydration (Mitchell et al., 1994;
8 Robertson, 1974). Hence, there is considerable scientific interest in understanding factors that enhance
9 fluid retention and assist with rehydration after exercise.

10 When consumed without food and matched for volume, nutrient dense beverages (e.g. milk and milk-
11 based beverages) appear to promote greater fluid retention compared to water and carbohydrate-
12 electrolyte solutions (Desbrow et al., 2014; Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al.,
13 2008). The effectiveness of milk as a rehydration solution has been attributed to a number of its
14 constituents (i.e. sodium (Merson et al., 2008; Shirreffs & Maughan, 1998), carbohydrate (Osterberg et
15 al., 2009), and protein (Hobson & James, 2015; James et al., 2014; James et al., 2012)), which are
16 believed to delay gastric emptying and/or attenuate changes in P_{OSM} , reducing the degree of fluid induced
17 diuresis (Calbet & MacLean, 1997; Clayton et al., 2014; Murray et al., 1999; Vist & Maughan, 1995).

18 Typically, post-exercise rehydration studies control drinking rate by prescribing fixed volumes of
19 beverages within standardised time periods. In contrast, active individuals may consume fluids at
20 different rates, which is likely to influence nutrient delivery and consequently, fluid retention. To date,
21 only two studies have investigated the influence of drinking rate on fluid recovery (Jones et al., 2010;
22 Kovacs et al., 2002). The initial investigation failed to detect differences in fluid retention when a
23 carbohydrate-electrolyte beverage was consumed over 3 h ($79\pm6\%$) compared to 5 h ($82\pm5\%$) following
24 exercise-induced dehydration (3.0% body mass (BM) loss). In contrast, Jones et al., (2010) reported
25 significantly greater retention when water was consumed over a 4 h ($75\pm12\%$) compared to a 1 h

(55±18%) drinking period following an exercise-induced 2.0% BM loss. The explanation for the equivocal findings may relate to the subtle differences in the drinking rates and/or the use of beverages with different nutrient profiles (and hence osmolalities). Furthermore, when consumed *ad libitum*, individuals typically ingest the largest volume of fluid within the first 30 min following exercise (Baguley et al., 2016). To date, the effect of drinking rate on the retention of fluid from beverages with contrasting nutrient profiles has not been systematically examined. In addition, no previous investigation has compared a conservative drink pattern to a rapid ingestion rate (e.g. large volumes consumed in ~30 min), which may reflect the actual behavior of individuals following exercise.

Therefore, the aim of the current study was to investigate the effect of rapid vs slower drinking rates on fluid retention using beverages with contrasting nutrient profiles (milk vs. water). It was hypothesized that the fluid retained from the consumption of a nutrient dense beverage would be unaffected by drinking rate; and that slower intake of a hypotonic beverage would enhance subsequent fluid retention.

Methods

Overview of study designs

This investigation was intended to systematically explore the effect of drinking rate on subsequent fluid recovery. The investigation was conducted in two parts, with the results from Part A used to inform the design of Part B. Part A explored the impact of drinking rates of different beverages (milk and water) on fluid retention. In Part B, further exploration of different drinking rates was performed. In addition, the trials were conducted in separate laboratories (Part A - Australia, Part B - Scotland). All participants were fully informed of the nature and possible risks of the investigations before providing written informed consent. The investigation was approved by the Griffith University and University of Stirling's Human Ethics Committees and the procedures were conducted in accordance with the principles outlined by the declaration of Helsinki.

Participant characteristics

In Part A thirteen healthy males volunteered to take part. However, one participant was unable to continue with the study after completing the first trial for reasons unrelated to the study (i.e. work commitments). Consequently, twelve male participants (age: 23.5±5.3 y; height: 179±6 cm; BM: 77.3±9.6 kg; maximal oxygen consumption ($\dot{V}O_{2peak}$): 43.1±6.4 mL·kg⁻¹·h⁻¹ (Mean±SD)) completed four experimental trials.

In Part B fourteen healthy participants volunteered to take part. However, one participant withdrew from the study due to external factors and one participant's data was excluded because they could not achieve the required level of dehydration. Consequently, twelve (9 males and 3 females) participants (age: 28.3±6.3 y; height: 176±11 cm; BM 74.0±10.2 kg; $\dot{V}O_{2peak}$: 50.6±7.6mL·kg⁻¹·h⁻¹) completed four experimental trials.

Study designs

A schematic representation of the experimental protocols is displayed in Figure 1. Both parts utilised a repeated-measures experimental design, involving 4 experimental trials; each separated by a minimum of 5 d. For all trials, participants lost ~2.0% BM through intermittent cycle exercise before cooling down and beginning a rehydration period in which different treatment interventions were examined (Part A, water or milk ingested over 30 or 90 min; Part B, water ingested over 15, 45, or 90 min with either the 15 or 45 min trial repeated). An incomplete Latin square design was used to counterbalance the order of treatments.

Preliminary requirements

Participants undertook an incremental test to exhaustion on a cycle ergometer. The protocol began at 100 W, and increased in 50 W increments every 2.5 min until volitional exhaustion, with participant's breath sampled continuously via a calibrated gas analysis system (Part A: Medgraphics Ultima, USA; Part B: Servomex Group Ltd, United Kingdom). The test was used to determine $\dot{V}O_{2peak}$ and maximum heart rate (HR_{max}), with these values used to guide the prescription of exercise intensity for the experimental trials.

Participants were instructed to abstain from caffeine (12 h), alcohol (24 h) and moderate to strenuous exercise (12 h) before all trials. During the 24 h period preceding the first trial, individuals completed a

food and beverage diary. They were also instructed to drink 500 mL of water at least 2 h before arrival at the laboratory (to assist with hydration) and abstain from all food and fluid (excluding water) after 21:00 h. Individuals were then instructed to repeat these behaviors prior to all subsequent experimental trials.

Experimental procedures

Participants arrived at the laboratory between 05:30 and 08:00 h and verbally acknowledged compliance to the pre-experimental conditions. A urine sample was taken for determination of hydration status (Part A: urine specific gravity (U_{SG}) (Palette Digital Refractometer, ATAGO, USA) and Part B: urine osmolality (U_{OSM}) (Löser Osmometer, Camlab, UK). If participants recorded a $U_{SG} \geq 1.024$ (Sommerfield et al., 2016) or U_{OSM} of $>700 \text{ mOsm} \cdot \text{kg}^{-1}$ (Sawka et al., 2007) they were considered hypohydrated. In Part A, hypohydrated participants were required to consume 600 mL of plain water over 5 min, before providing a second urine sample 30-60 min later. If this urine sample achieved the thresholds for euhydration the participants continued with the trial (this practice was then replicated on all subsequent trials). If the threshold value was not reached within the 60 min period the trial was rescheduled. Participants then rested in a seated position for 5 min prior to venepuncture of a forearm vein. Following this initial blood collection, participants were provided with a standardised breakfast in a quantity relative to BM ($20 \text{ kJ} \cdot \text{kg}^{-1}$ and $1 \text{ g CHO} \cdot \text{kg}^{-1}$) that consisted of raisin toast, strawberry jam and fruit juice (200 g) before completing a questionnaire on GI subjective symptoms, voiding their bladder and obtaining a baseline nude BM measurement (Part A: A&D Company Ltd, Tokyo, Japan, to nearest 20 g; Part B: Marsden, Rotherham, United Kingdom, to nearest 10 g).

Exercise-induced dehydration

After completing a brief standardised warm up, participants began cycling in a warm environment (Part A: $25.2 \pm 0.8^\circ \text{C}$ and $84 \pm 11\% \text{ RH}$, Part B: $26.4 \pm 0.7^\circ \text{C}$ and $38 \pm 5\% \text{ RH}$). Individuals commenced exercise at a workload corresponding to $\sim 65\%$ of HR_{max} . Intensity was recorded by an investigator and replicated on all subsequent trials. Following 50 min of cycling, participants BM was measured. A BM loss of $<1.8\%$ from baseline required participants to continue exercising in 10 min bouts until a BM loss $\geq 1.8\%$ was

100 achieved. Following exercise, dehydrated participants rested in a seated position for 15 min prior to
101 having a cool shower. Afterwards, participants dried themselves thoroughly, before a cannula was
102 inserted into a forearm vein and a blood sample obtained. Participants then emptied their bladder and
103 provided a urine sample before a final nude BM measure was recorded to determine total fluid loss (30
104 min post-exercise).

105 ***Post-exercise fluid replacement***

106 In Part A, water or low fat cow’s milk (Maleny Dairies, Queensland, Australia; 210 kJ Energy, 5.3 g
107 CHO, 4.0 g Protein, 1.4 g Fat, 48 mg Na⁺·100 mL⁻¹) were ingested in a quantity equal to 100% of the
108 volume of sweat lost during exercise. The fluid volume was ingested in six equal aliquots spread evenly
109 over either a 30 or 90 min period, resulting in the beverage treatments: Water 30 min (W30), Water 90
110 min (W90), Milk 30 min (M30), and Milk 90 min (M90). Participants were instructed to consume each
111 aliquot at an even pace over 5 or 15 min according to the relevant drinking rate. In Part B, water in a
112 quantity equal to 100% of the volume of sweat lost during exercise was ingested. The volume was
113 provided in three aliquots spaced evenly over either a 15, 45 or 90 min drinking period, resulting in the
114 following beverage treatments: Water 15 min (DR15); Water 45 min (DR45); and Water 90 min (DR90).
115 To assess within individual variation, participants in part B repeated either the DR15 or DR45 trial. To
116 assess inter-site variation W90 (Part A) was compared to DR90 (Part B).

117 A 3 h rehydration monitoring period (from the commencement of drinking) was applied to all trials.
118 Observations were made every hour and included measures of nude BM, urine and plasma measures of
119 hydration status. In addition, subjective measures of bloatedness, fullness and thirst were recorded. All
120 measurements were obtained while participants remained seated.

121 ***Body mass and fluid retention***

122 BM change (estimate of fluid loss) was calculated by subtracting the post-exercise BM (post-void) from
123 the pre-exercise BM. Net BM change was calculated by subtracting the 3 h BM measurement from the

pre-exercise BM. Percent fluid retention at the conclusion of the observation period was calculated by the following equation:

$$\text{Fluid Retained (\%)} = 100 \times \frac{(\text{Total beverage ingested (g)} - \text{Total urine output (g)})}{\text{Total beverage ingested (g)}}$$

Urine and blood collection, storage and analysis

Additional urine sampling was performed at pre-exercise, post-exercise (immediately pre-drinking), immediately post-drinking and then at 120 min and 180 min after the start of drinking. At each of these urine collection points, participants completely voided their bladder into an empty container for subsequent measures of urine volume. Total urine loss was calculated from the accumulated urine output in the period from the commencement of drinking until the end of the observation period. A sample of urine was retained for determination of urine osmolality. Blood sampling was performed at pre-exercise, post-exercise (immediately pre-drinking), immediately post-drinking and then at 120 min and 180 min after the start of drinking for the determination of P_{OSM} . Participants remained seated prior to a 5 mL blood sample being drawn from an antecubital vein. All samples were collected into EDTA pre-treated vacutainers and centrifuged at room temperature for 10 min at $\sim 1350 \times g$. Plasma was analysed in duplicate on a calibrated freezing-point depression osmometer (Part A: Osmomat 030, Germany and Part B: Löser osmometer, Camlab, UK). Cannulas were kept patent by flushing sterile saline (2 mL of 0.9% NaCl; Becton Dickson, NJ, USA) on completion of each sample (with an equivalent volume of blood initially discarded before collection of subsequent samples).

Subjective measures

Subjective ratings of bloatedness, fullness and thirst were recorded on separate 100 mm visual analog scales, with 0 mm representing 'not at all' and 100 mm representing 'extremely'. Scales were administered via a computerized modifiable software program (Marsh-Richard et al., 2009).

146 **Statistical analyses**

147 Statistical analyses were performed using SPSS Statistics for Windows, Version 22 (SPSS Inc., IBM,
148 Chicago, IL). All measures were examined for normality and sphericity using the Shapiro-Wilk test
149 ($p>0.05$) and Mauchly's test ($p>0.05$), respectively. Where assumptions of sphericity in repeated-
150 measures analyses were violated, the Greenhouse-Geisser statistic was applied. One-way repeated-
151 measures analysis of variance (ANOVA) were performed to verify that pre-trial conditions and exercise-
152 induced fluid loss did not differ across trials. For Part A, a three-factor (i.e. Beverage x Rate x Time)
153 repeated-measures ANOVA was used to compare main outcomes; two-factor (i.e. Beverage x Rate)
154 repeated-measures ANOVA were conducted to compare total fluid retention and net BM changes across
155 treatments. Pairwise comparison (Bonferroni) were performed where significant main effects were
156 present. For Part B, two-factor (i.e. Rate x Time) repeated-measures ANOVA were used to compare
157 outcomes between the different beverage ingestion rates. Paired t -tests or Wilcoxon tests were used where
158 appropriate to conduct post-hoc comparisons on significant interaction effects. An adjusted-alpha (i.e.
159 $p=0.05$ divided by the number of tests performed) was used to account for multiple comparisons. The
160 test-retest reliability was calculated as a coefficient of variation (CV%) using the traditional method and
161 any difference in responses between sites was assessed using an unpaired t -test. Statistical significance
162 was accepted at $p<0.05$. All data are reported as Mean \pm SD, unless stated as Mean \pm SEM.

163 **Results**

164 **Standardisation procedures**

165 All participants reported compliance with the standardisation procedures in the 24 h prior to arriving at
166 the laboratory. In Part A, two participants were administered water (600 mL) due to a pre-exercise U_{SG}
167 ≥ 1.024 on Trial 1; this practice was repeated on all subsequent trials to ensure consistency. The remaining
168 participants had a $U_{SG}<1.024$ at the commencement of each trial. Exercise duration and pre-exercise
169 values for BM, U_{SG} and P_{OSM} were similar across all treatments, and did not differ significantly by trial
170 order ($p>0.05$). Exercise-induced BM loss differed significantly ($p<0.01$) by trial order (Trial 1:

1.54±0.26 kg; Trial 2: 1.44±0.28 kg; Trial 3: 1.41±0.31 kg; Trial 4: 1.38±0.32 kg); however, counterbalancing ensured that mass loss was similar across treatment conditions (Table 1).

In Part B, exercise duration and pre-exercise values for BM, U_{OSM} , P_{OSM} , and exercise induced BM loss were similar across all treatments (Table 1); and did not differ significantly by trial order ($p>0.05$).

Urine output and fluid retention

In Part A, cumulative urine output was greater with water than with milk at 120 min (398±190 vs. 139±44 g) and 180 min (592±248 vs. 224±70 g) after the start of drinking ($p<0.01$; Figure 2A). A significant effect of beverage was observed on fluid retention (W30: 56.5±16.1%; W90: 59.7±19.9%; M30: 82.9±6%; M90: 84.9±7%) with the proportion of ingested fluid retained lower with water than milk (58.1±15.6 vs. 83.9±6.1%, $p<0.01$). No other significant differences were observed in either analysis.

In Part B, a similar cumulative urine output response was observed when water was ingested at DR15, DR45 and DR90 rates. Three hours after the start of the drinking period, cumulative urine output was lower for the DR90 trial (602±183 g) compared to the DR45 (750±373 g) and DR15 (754±230 g) trials, but this did not reach statistical significance ($p>0.05$). The mean difference (95% CI) between DR15 and DR90 was 7.4(1.2-13.6)%, equivalent to 152 (43-260) mL (Figure 2B). Fluid retention was significantly higher ($p<0.05$) on the DR90 trial (57.1±12.9%) compared to the DR15 trial (49.7±11.0%), but these trials were not different ($p>0.05$) to DR45 (51.6±19.8%).

Net fluid balance

In Part A, all experimental trials concluded with participants in a state of negative net fluid balance 180 min after the ingestion period started (Part A: W30: -0.68±0.31 L; W90: -0.61±0.25 L; M30: -0.27±0.07 L; M90: -0.28±0.08 L; Figure 3A). Post hoc comparisons revealed that milk ingestion led to less negative fluid balance compared to water at 120 min (-0.40±0.19 vs. -0.14±0.04 L, $p=0.001$) and 180 min (-0.64±0.27 vs. -0.28±0.07 L, $p<0.001$) after drinking started. Fluid balance was also less negative

194 immediately post-drinking for the 30 min compared to the 90 min drinking trials (-0.14 ± 0.08 vs.
195 0.04 ± 0.03 L, $p<0.001$), since participants had less time to produce urine on these trials.

196 In Part B, all experimental trials concluded with participants in a state of negative net fluid balance
197 (DR15: -0.75 ± 0.23 L; DR45: -0.75 ± 0.37 L; DR90: -0.60 ± 0.18 L; Figure 3B). No differences were
198 observed between trials.

199 ***Plasma osmolality***

200 In Part A, the consumption of water decreased P_{OSM} compared to milk at the cessation of drinking (291 ± 4
201 vs. 298 ± 5 mOsm \cdot kg $^{-1}$, $p<0.001$), but this effect was not evident by 180 min (Water: 290 ± 2 mOsm \cdot kg $^{-1}$;
202 Milk: 293 ± 4 mOsm \cdot kg $^{-1}$, $p=0.033$). P_{OSM} did not differ significantly as a result of the fluid ingestion rate
203 at any point ($p>0.05$).

204 In Part B, a drinking rate by time interaction was not evident for P_{OSM} . Plasma osmolality 180 minutes
205 after start of drink ingestion did not differ significantly as a result of the fluid ingestion rate (DR15:
206 304 ± 2 mOsm \cdot kg $^{-1}$; DR45: 302 ± 3 mOsm \cdot kg $^{-1}$; DR90: 303 ± 5 mOsm \cdot kg $^{-1}$, $p>0.05$).

207 ***Subjective measures***

208 In Part A, analysis for bloatedness, fullness, and thirst ratings identified a significant effect of time on
209 each variable ($p<0.01$). A significant effect of beverage was also observed for fullness ($p=0.022$). For
210 bloatedness and fullness there were significant time x beverage interaction effects (bloatedness: $p=0.014$;
211 fullness $p<0.01$). Post hoc comparisons revealed that the 30 min drinking protocol increased feelings of
212 bloatedness ($p<0.01$) and decreased feelings of thirst ($p<0.01$) immediately after drinking compared to
213 the 90 min protocol. The consumption of milk increased feelings of fullness immediately after drinking
214 ($p<0.01$) and at 120 min ($p<0.01$), compared to the consumption of water. No other significant
215 differences were observed.

216 In Part B, perceived bloatedness and fullness were significantly higher immediately after drinking on the
217 DR15 trials compared to the DR45 and DR90 drinking rates ($p<0.01$), but were not different at

subsequent time points up to 180 min. No other significant differences were observed at any other time point (Figure 4).

Reliability and inter-lab repeatability

The CV% of test re-test reliability between duplicate trials on DR15 and DR45 ingestion rates (Part B) was 17%. Data from repeated trials was not significantly different (Table 2). The fluid retention on 90 min water rate trials (Part A: W90 and Part B: DR90) was not significantly different between testing sites (W90: $59.7 \pm 19.9\%$; DR90: $57.1 \pm 12.9\%$, $p=0.73$).

Discussion

This two-part study explored the effect of drinking rate on fluid retention of different beverages following exercise-induced dehydration. In keeping with our hypothesis, Part A observed that drinking milk resulted in greater fluid retention than water during a 3 h recovery period. This effect was not influenced by drinking rate (i.e. 30 vs. 90 min). Consequently, Part B assessed retention of water consumed over alternative drinking rates (i.e. 15 vs. 45 vs. 90 min), as well as the day-to-day variation in post-exercise fluid retention. Part B, indicated that the 15 min drinking protocol led to a significant reduction in fluid retention compared to the 90 min drinking protocol. However, the magnitude of the effect was within the CV% of the repeated trials (17%). Thus, findings from this study suggest the influence of drinking rate on post-exercise fluid recovery is small and that the nutrient composition of a beverage has a more pronounced impact on fluid retention than the beverage ingestion rate.

Only two studies have previously investigated the influence of drinking rate on fluid recovery (Jones et al., 2010; Kovacs et al., 2002). Results from these studies are contradictory, with only one investigation (Jones et al., 2010) identifying an influence of drinking rate on fluid retention. Jones et al., (2010) had participants ingest water at $1.61 \text{ L}\cdot\text{h}^{-1}$ vs. $0.40 \text{ L}\cdot\text{h}^{-1}$. Kovacs et al., (2002) had participants ingest a carbohydrate-electrolyte sports drink at a maximum rate of $1.32 \text{ L}\cdot\text{h}^{-1}$ in the first hour, with an average rate over 3 hours of $0.77 \text{ L}\cdot\text{h}^{-1}$ and compared this to fluid retention with a slow drinking rate of $0.53 \text{ L}\cdot\text{h}^{-1}$ over 5 h. These fluid consumption patterns are slower than those observed when individuals drink *ad*

libitum post-exercise (e.g. with drinking rates in the first 30 min exceeding 2 L·h⁻¹, Baguley et al., 2016). The present study attempted to assess drinking rates across a broader range (5.84 L·h⁻¹ (1.46 L in 15 min) to 0.95 L·h⁻¹ (1.42 L in 90 min)) to elucidate effects on fluid retention. We observed little impact of contrasting drinking rates on fluid retention with water. In fact, the only difference noted in Part B (DR15 vs. DR90) was within the CV% of the method.

The current findings suggest that the nutrient profile of different beverages have a greater impact on fluid retention than ingestion rate. Indeed, when consumed exclusively and matched for volume, milk beverages promote greater fluid retention than water at rest (Maughan et al., 2016) and during the post-exercise period (Seery & Jakeman, 2016; Shirreffs et al., 2007; Watson et al., 2008). These effects may be mediated by the composition of milk (whey/casein protein), electrolyte content and insulin response to carbohydrate/protein delivery. In addition, the electrolyte content of milk (Shirreffs et al., 2007) and insulin mediated impacts on renal water transport (Magaldi et al., 1994) both have the potential to enhance fluid retention.

In a practical sense post-exercise, athletes typically consume fluids ad libitum and the beverage choice, drinking rate and total volume consumed are determined by many factors, including prior exercise (intensity, duration and type), environmental conditions, thirst, palatability, gastrointestinal tolerance, drink availability, exercise commitments and other, unrelated dietary goals (Minehan et al., 2002; Passe et al., 2000). The rapid consumption of large volumes of milk or water during the immediate post-exercise period may be poorly tolerated by some individuals. However, the range of subjective responses to our most rapid drinking rates highlights individual differences in tolerance. For those who drink beverages rapidly in the immediate post-exercise period, the rates examined in the present study do not appear to compromise fluid retention when a fixed volume is provided and may facilitate the consumption of other fluids after completing a “prescribed” volume of a beverage. Conversely, it is not known whether rapid beverage ingestion compromises subsequent voluntary fluid consumption in ad libitum drinking scenarios due to an action on thirst response mediated via the gut-brain axis (Zimmerman et al., 2019).

Several methodological limitations require acknowledgement. Firstly, this study did not employ a direct measure of gastric emptying. Hence, while greater fluid retention was achieved during Milk trials, the distribution of the retained fluid (e.g. within the GI tract (as potentially indicated by higher “fullness” ratings) as opposed to vascular space) and therefore physiological relevance of this fluid retention remains unknown. In addition, the recovery period for this study (3 h from the start of drinking) was shorter than previous work in this area (typically ≥ 4 h), which may have resulted in small volumes of uncaptured fluid losses in response to the differences in drinking strategy. The decision to shorten the duration of the observation was based on a number of factors; (1) the relatively small volumes of urine seen beyond 90 min following the cessation of drinking in our previous study (Desbrow et al 2014), (2) the smaller volume of fluid being ingested (100% vs. 150% fluid replacement), (3) the practical relevance of 4 h observation, given that many individuals are likely to eat/drink within this period of time and (4) our previous study (Maughan et al., 2016) demonstrated the pattern of response in cumulative urine output and calculated hydration index to ingested drinks was observed to be similar at 2 h post-drinking and 4 h post-drinking.

Conclusion

This study suggests that drinking more rapidly does not compromise post-exercise fluid retention following moderate intensity exercise in recreationally active participants. This observation was consistent between different testing sites and across different drinking rates. Laboratory informed findings suggest that beverage composition is more influential than fluid ingestion rate in determining post-exercise fluid retention.

Acknowledgments

The authors declare no conflicts of interest.

B. Desbrow and S. D. R. Galloway conceived the project. B. Desbrow, S. D. R. Galloway, G. R. Cox, C. Irwin, N. Rodriguez-Sanchez, D. McCartney, P. Rodriguez-Giustiniani and L. Sayer developed the overall research plan. L. Sayer, D. McCartney, C. Irwin, N. Rodriguez-Sanchez and P. Rodriguez-

Giustiniani conducted the research and analysed the samples. B. Desbrow, S. D. R. Galloway, C. Irwin, N. Rodriguez-Sanchez, D. McCartney and L. Sayer performed the statistical analysis. B. Desbrow, S. D. R. Galloway, G. R. Cox, C. Irwin, N. Rodriguez-Sanchez, D. McCartney, P. Rodriguez-Giustiniani and L. Sayer wrote the paper. All the authors approved the final version of the paper.

References

Baguley, B. J., Zilujko, J., Leveritt, M. D., Desbrow, B., & Irwin, C. (2016). The effect of ad libitum consumption of a milk-based liquid meal supplement vs. a traditional sports drink on fluid balance after exercise. *International Journal of Sport Nutrition and Exercise Metabolism*, 26(4), 347-355.

Baker, L. B., & Jeukendrup, A. E. (2014). Optimal composition of fluid-replacement beverages. *Comprehensive Physiology*. 4(2), 575-620.

Borg, G. A. (1973). Perceived exertion: a note on “history” and methods. *Medicine and Science in Sports*, 5(2), 90-93.

Calbet, J., & MacLean, D. (1997). Role of caloric content on gastric emptying in humans. *The Journal of Physiology*, 498(2), 553-559.

Clayton, D. J., Evans, G. H., & James, L. J. (2014). Effect of drink carbohydrate content on postexercise gastric emptying, rehydration, and the calculation of net fluid balance. *International Journal of Sport Nutrition and Exercise Metabolism*, 24(1), 79-89.

Desbrow, B., Jansen, S., Barrett, A., Leveritt, M. D., & Irwin, C. (2014). Comparing the rehydration potential of different milk-based drinks to a carbohydrate–electrolyte beverage. *Applied Physiology, Nutrition, and Metabolism*, 39(12), 1366-1372.

Evans, G. H., James, L. J., Shirreffs, S. M., & Maughan, R. J. (2017). Optimizing the restoration and maintenance of fluid balance after exercise-induced dehydration. *Journal of Applied Physiology*, 122(4), 945-951.

Evans, G. H., Shirreffs, S. M., & Maughan, R. J. (2009). Postexercise rehydration in man: the effects of carbohydrate content and osmolality of drinks ingested ad libitum. *Applied Physiology, Nutrition,*

- 318 *and Metabolism*, 34(4), 785-793.
- 319 Garth, A. K., & Burke, L. M. (2013). What do athletes drink during competitive sporting activities?
- 320 *Sports Medicine*, 43(7), 539-564.
- 321 Goulet, E. D., Lamontagne-Lacasse, M., Gigou, P.-Y., Kenefick, R. W., Ely, B. R., & Cheuvront, S.
- 322 (2010). Pre-exercise Hypohydration Effects On Jumping Ability And Muscle Strength, Endurance
- 323 And Anaerobic Capacity: A Meta-analysis: 1681. *Medicine & Science in Sports & Exercise*, 42(5),
- 324 362.
- 325 Hobson, R., & James, L. (2015). The addition of whey protein to a carbohydrate-electrolyte drink does
- 326 not influence post-exercise rehydration. *Journal of Sports Sciences*, 33(1), 77-84.
- 327 James, L. J., Gingell, R., & Evans, G. H. (2012). Whey protein addition to a carbohydrate-electrolyte
- 328 rehydration solution ingested after exercise in the heat. *Journal of Athletic Training*, 47(1), 61-66.
- 329 James, L. J., Mattin, L., Aldiss, P., Adebishi, R., & Hobson, R. M. (2014). Effect of whey protein isolate
- 330 on rehydration after exercise. *Amino Acids*, 46(5), 1217-1224.
- 331 Jones, E. J., Graham, J., Newcomb, T., & Frischman, N. (2010). Effects of Bolus vs. Metered
- 332 Rehydration Rates on Fluid Retention and Hydration Efficiency using 150% Fluid Replacement:
- 333 2290. *Medicine & Science in Sports & Exercise*, 42(5), 575.
- 334 Kovacs, E. M., Schmahl, R. M., Senden, J. M., & Brouns, F. (2002). Effect of high and low rates of fluid
- 335 intake on post-exercise rehydration. *International Journal of Sport Nutrition and Exercise*
- 336 *Metabolism*, 12(1), 14-23.
- 337 Magaldi, A., Cesar, K., & Yano, Y. (1994). Effect of insulin on water and urea transport in the inner
- 338 medullary collecting duct. *American Journal of Physiology-Renal Physiology*, 266(3), F394-F399.
- 339 Marsh-Richard, D. M., Hatzis, E. S., Mathias, C. W., Venditti, N., & Dougherty, D. M. (2009). Adaptive
- 340 Visual Analog Scales (AVAS): a modifiable software program for the creation, administration, and
- 341 scoring of visual analog scales. *Behavior Research Methods*, 41(1), 99-106.
- 342 Maughan, R. J., Watson, P., Cordery, P. A., Walsh, N. P., Oliver, S. J., Dolci, A., Galloway, S. D. (2016).
- 343 A randomized trial to assess the potential of different beverages to affect hydration status:

344 development of a beverage hydration index. *The American Journal of Clinical Nutrition*, 103(3),
345 717-723.

346 Merson, S. J., Maughan, R. J., & Shirreffs, S. M. (2008). Rehydration with drinks differing in sodium
347 concentration and recovery from moderate exercise-induced hypohydration in man. *European*
348 *Journal of Applied Physiology*, 103(5), 585.

349 Minehan, M. R., Riley, M. D., & Burke, L. M. (2002). Effect of flavor and awareness of kilojoule content
350 of drinks on preference and fluid balance in team sports. *International Journal of Sport Nutrition*
351 *and Exercise Metabolism*, 12(1), 81-92.

352 Mitchell, J. B., Grandjean, P. W., Pizza, F. X., Starling, R. D., & Holtz, R. W. (1994). The effect of
353 volume ingested on rehydration and gastric emptying following exercise-induced dehydration.
354 *Medicine and Science in Sports and Exercise*, 26(9), 1135-1143.

355 Murray, R., Bartoli, W., Stofan, J., Horn, M., & Eddy, D. (1999). A comparison of the gastric emptying
356 characteristics of selected sports drinks. *International Journal of Sport Nutrition*, 9(3), 263-274.

357 Osterberg, K. L., Pallardy, S. E., Johnson, R. J., & Horswill, C. A. (2009). Carbohydrate exerts a mild
358 influence on fluid retention following exercise-induced dehydration. *Journal of Applied*
359 *Physiology*, 108(2), 245-250.

360 Passe, D., Horn, M., & Murray, R. (2000). Impact of beverage acceptability on fluid intake during
361 exercise. *Appetite*, 35(3), 219-229.

362 Robertson, G. L. (1974). Vasopressin in osmotic regulation in man. *Annual Review of Medicine*, 25(1),
363 315-322.

364 Savoie, F.A., Kenefick, R. W., Ely, B. R., Cheuvront, S. N., & Goulet, E. D. (2015). Effect of
365 hypohydration on muscle endurance, strength, anaerobic power and capacity and vertical jumping
366 ability: a meta-analysis. *Sports Medicine*, 45(8), 1207-1227.

367 Sawka, M. N., Burke, L. M., Eichner, E. R., Maughan, R. J., Montain, S. J., & Stachenfeld, N. S. (2007).
368 American College of Sports Medicine position stand. Exercise and fluid replacement. *Medicine and*
369 *Science in Sports and Exercise*, 39(2), 377-390.

- 370 Seery, S., & Jakeman, P. (2016). A metered intake of milk following exercise and thermal dehydration
371 restores whole-body net fluid balance better than a carbohydrate–electrolyte solution or water in
372 healthy young men. *British Journal of Nutrition*, 116(6), 1013-1021.
- 373 Shirreffs, S. M., & Maughan, R. J. (1998). Volume repletion after exercise-induced volume depletion in
374 humans: replacement of water and sodium losses. *American Journal of Physiology-Renal*
375 *Physiology*, 274(5), F868-F875.
- 376 Shirreffs, S. M., Watson, P., & Maughan, R. J. (2007). Milk as an effective post-exercise rehydration
377 drink. *British Journal of Nutrition*, 98(1), 173-180.
- 378 Sommerfield, L. M., McAnulty, S. R., McBride, J. M., Zwetsloot, J. J., Austin, M. D., Mehlhorn, J. D.,
379 Utter, A. C. (2016). Validity of Urine specific gravity when compared to plasma osmolality as a
380 measure of hydration status in male and female NCAA collegiate athletes. *Journal of strength and*
381 *conditioning research/National Strength & Conditioning Association*, 30(8), 2219.
- 382 Vist, G. E., & Maughan, R. J. (1995). The effect of osmolality and carbohydrate content on the rate of
383 gastric emptying of liquids in man. *The Journal of Physiology*, 486(2), 523-531.
- 384 Watson, P., Love, T. D., Maughan, R. J., & Shirreffs, S. M. (2008). A comparison of the effects of milk
385 and a carbohydrate-electrolyte drink on the restoration of fluid balance and exercise capacity in a
386 hot, humid environment. *European Journal of Applied Physiology*, 104(4), 633-642.
- 387 Zimmerman, C. A., Huey, E. L., Ahn, J. S., Beutler, L. R., Tan, C. L., Kosar, S., Madisen, L. (2019). A
388 gut-to-brain signal of fluid osmolarity controls thirst satiation. *Nature*, 568(7750), 98-102

389 **Figure legends and footnotes**

390 **Figure 1.** Schematic of experimental protocol investigating the effect of drinking rate on fluid retention following
391 exercise.

392
393 **Figure 2.** Cumulative urine output before and after the test drink ingestion equal to the volume of sweat lost during
394 exercise. A = Part A (Water or Milk ingested over 30 or 90 min, Water 30 (W30); Water 90 (W90); Milk 30 (M30);
395 and Milk 90 (M90)), B = Part B (Water ingested over 15 (DR15), 45 (DR45) or 90 (DR90) mins). *a*, milk
396 significantly different to water. Values are Mean±SD.

397
398 **Figure 3.** Net fluid balance responses before and after the test drink ingestion equal to the volume of sweat lost
399 during exercise. A = Part A (Water or Milk ingested over 30 or 90 min, Water 30 (W30); Water 90 (W90); Milk 30
400 (M30); and Milk 90 (M90)), B = Part B (Water ingested over 15 (DR15), 45 (DR45) or 90 (DR90) mins). *a*, milk
401 significantly different to water; *b*, rapid drinking significantly different to metered drinking. Values are Mean±SD.

402
403 **Figure 4.** Subjective gastrointestinal ratings of bloatedness, fullness and thirst before and after test drink ingestion
404 equal to the volume of sweat lost during exercise. Part A = Panels A, B and C and Part B = Panels D, E and F. *a*,
405 milk significantly different to water; *b*, rapid drinking significantly different to metered drinking; *c*, fast ingestion
406 rate significantly different to slow ingestion rate. Values are Mean±SEM, where 0 represents ‘not at all’ and 100
407 represents ‘extremely much’ for each subjective feeling.

409 Table 1. Pre-trial conditions and impact of exercise-induced dehydration

| Part A | W30 | W90 | M30 | M90 | <i>p</i> -value |
|--|-------------|--------------|--------------|-------------|-----------------|
| Pre-Ex U _{SG} | 1.015±0.006 | 1.015±0.007 | 1.013±0.005 | 1.014±0.005 | 0.35 |
| Pre-Ex P _{OSM} (mOsm·kg ⁻¹) | 290±4 | 292±5 | 290±6 | 289±5 | 0.67 |
| Pre-Ex BM (kg) | 77.10±9.67 | 77.27±9.78 | 76.77±9.73 | 76.57±9.52 | 0.28 |
| Ex Duration (min) | 70±14 | 70±13 | 70±13 | 70±12 | 0.86 |
| BM Loss (kg) | 1.46±0.28 | 1.42±0.30 | 1.43±0.32 | 1.46±0.29 | 0.79 |
| BM Loss (%) | 1.9±0.3 | 1.9±0.4 | 1.9±0.4 | 1.9±0.3 | 0.82 |
| Part B | DR15 | DR45 | DR90 | | <i>p</i> -value |
| Pre-Ex U _{OSM} | 477±218 | 474±178 | 443±185 | | 0.76 |
| Pre-Ex P _{OSM} (mOsm·kg ⁻¹) | 303±5 | 302±3 | 302± 5 | | 0.36 |
| Pre-Ex BM (kg) | 71.60±9.90 | 71.54± 10.15 | 71.31± 10.08 | | 0.39 |
| Ex Duration (min) | 79±12 | 81±13 | 80± 11 | | 0.62 |
| BM Loss (kg) | 1.46±0.35 | 1.51± 0.33 | 1.45±0.32 | | 0.30 |
| BM Loss (%) | 2.0± 0.4 | 2.1±0.2 | 2.0±0.3 | | 0.61 |

410 BM: Body mass; Ex: Exercise; P_{OSM}: Plasma osmolality; U_{SG}: Urine specific gravity; U_{OSM}: Urine osmolality.
 411 Values are Mean±SD.

413 Table 2. Test-retest trial data (Part B: pooled from DR15 and DR45)

| | Initial Trial | Repeat Trial | <i>p-value</i> |
|--|---------------|--------------|----------------|
| Pre-Trial Conditions | | | |
| Pre-Ex U _{OSM} | 483±197 | 479±197 | 0.30 |
| Pre-Ex P _{OSM} (mOsm·kg ⁻¹) | 307±5 | 307±7 | 0.81 |
| Pre-Ex BM (kg) | 72.56±11.10 | 72.38±10.94 | 0.30 |
| Ex Duration (min) | 80.0±13.5 | 80.8±13.1 | 0.34 |
| BM Loss (kg) | 1.43±0.32 | 1.39±0.39 | 0.62 |
| Fluid Retention Data | | | |
| Cumulative urine output (g) | 792± 280 | 704±175 | 0.07 |
| U _{OSM} 180 min after drinking started (mOsm·kg ⁻¹) | 297±75 | 281±127 | 0.69 |
| P _{OSM} 180 min after drinking started (mOsm·kg ⁻¹) | 303±4 | 302±4 | 0.38 |
| Fluid retention (%) | 52.8±7.0 | 55.0±7.5 | 0.21 |

414 Values are mean±SD.

415

416

417

418

420 **Figure 1**
421
422

423
424

425
426
427
428
429
430
431
432
433
434
435
436

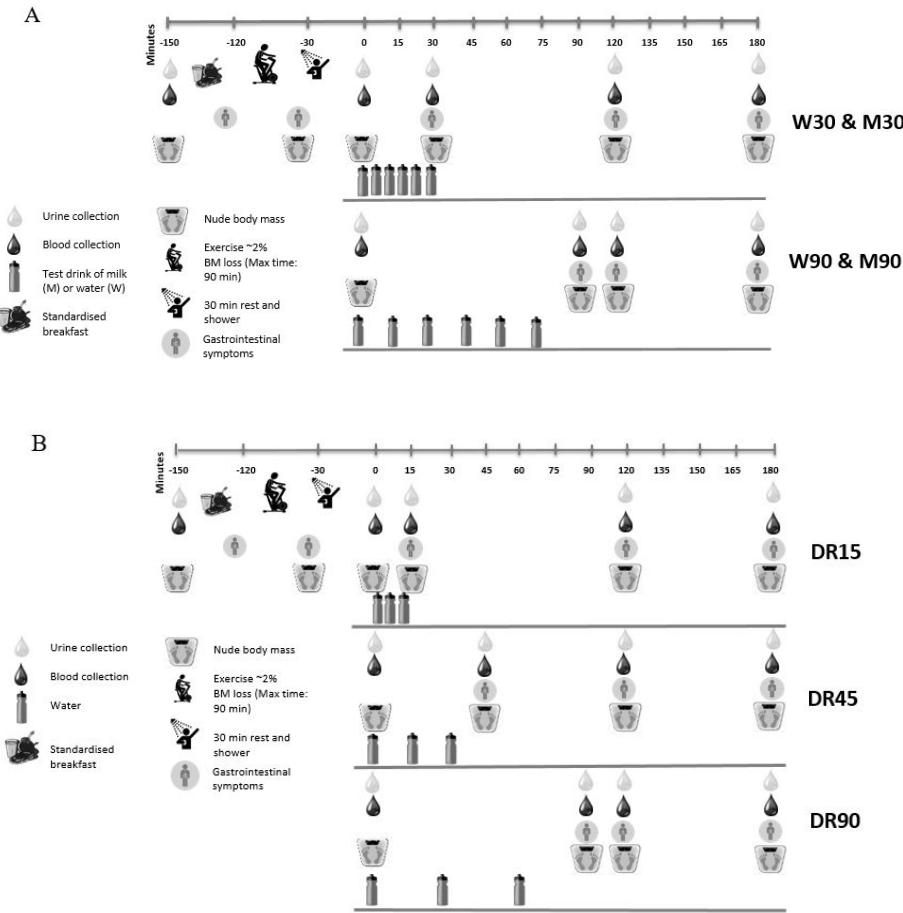
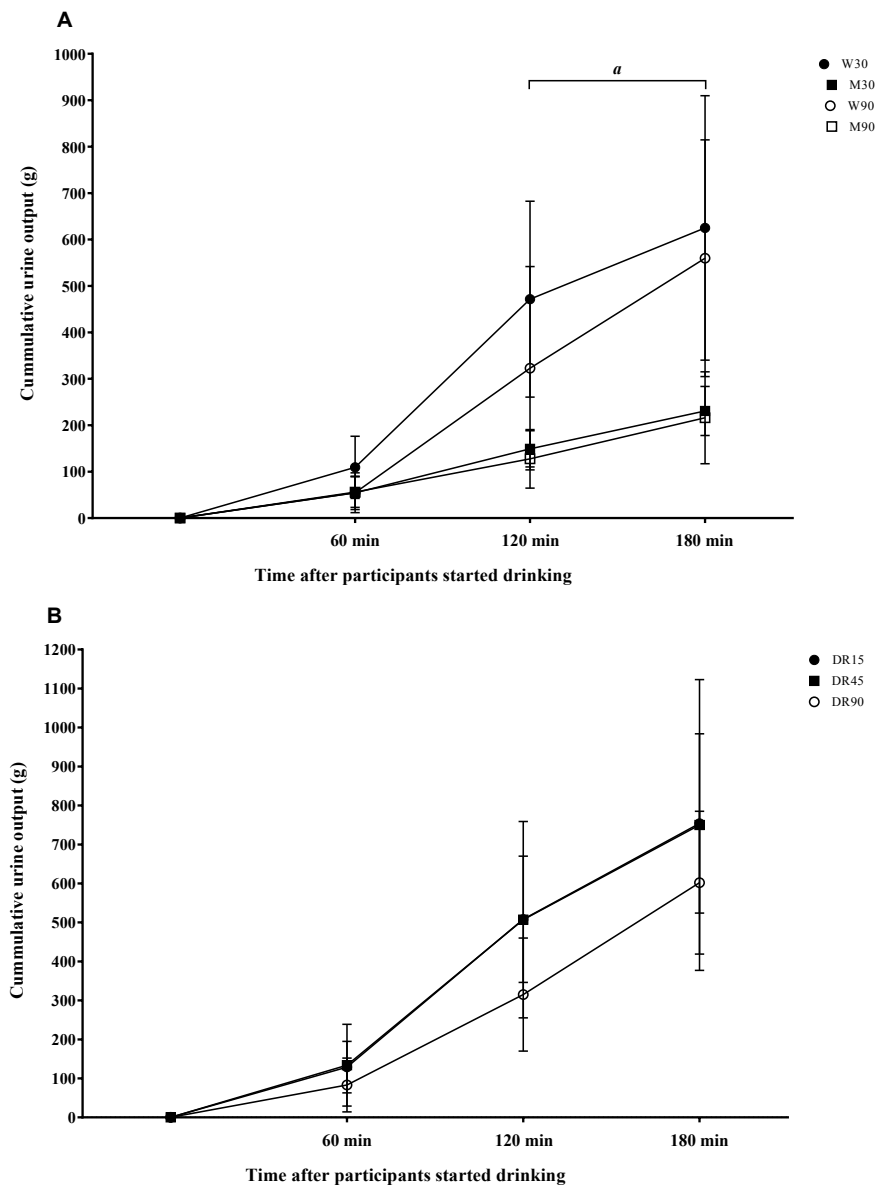
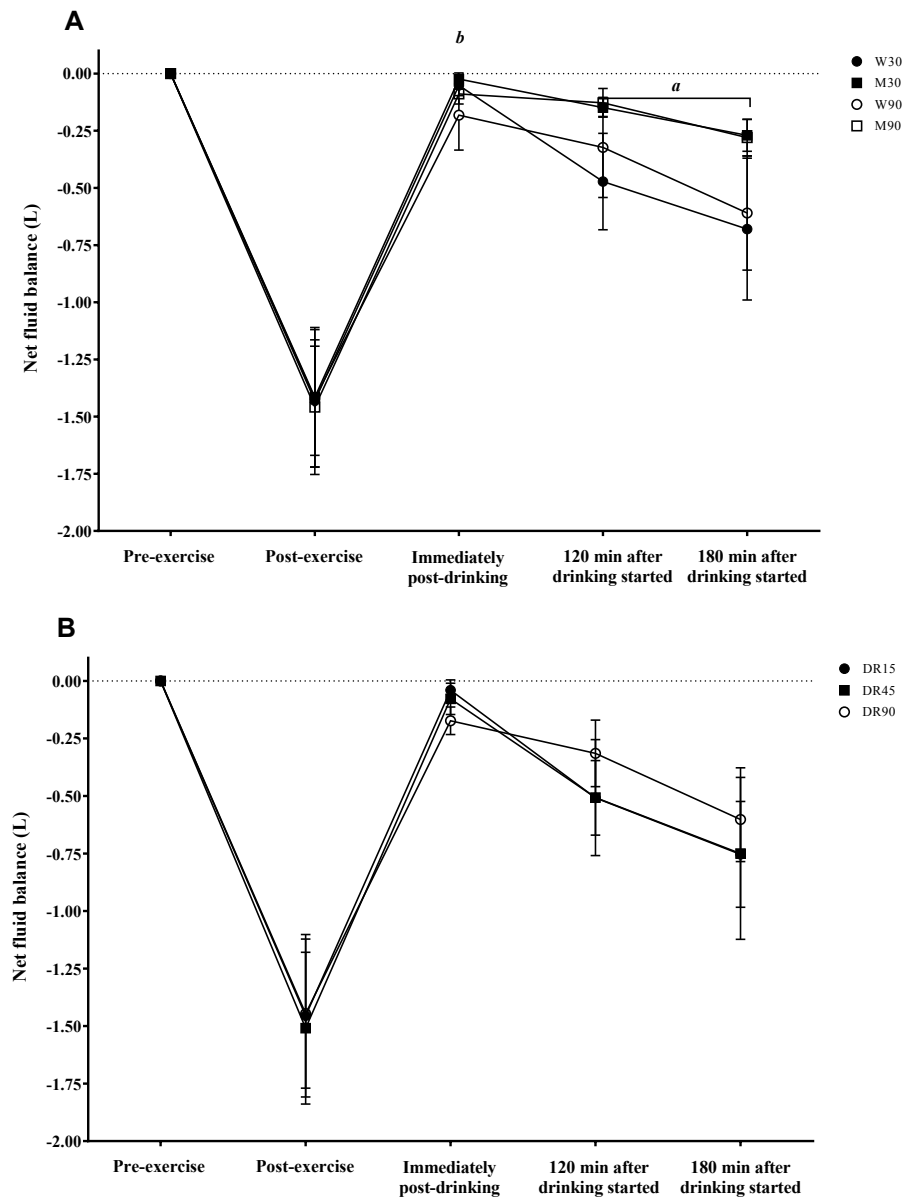


Figure 2

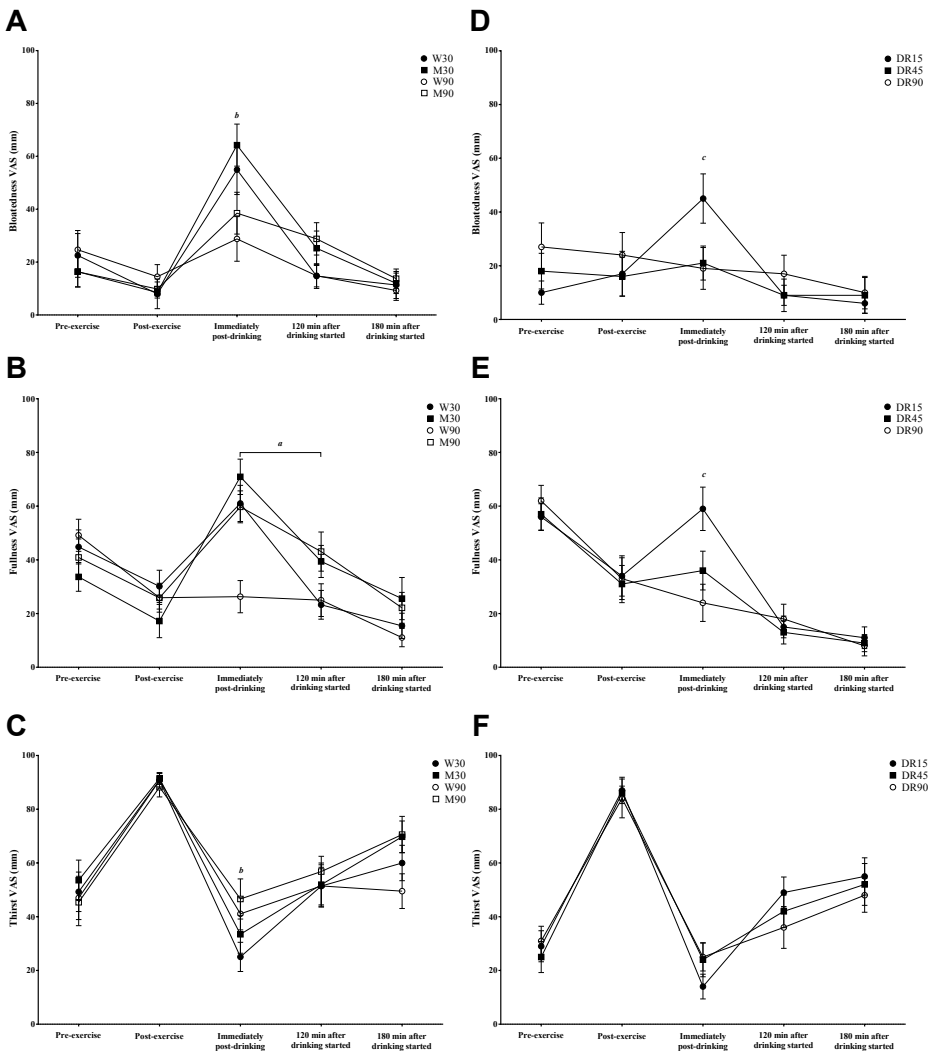


6

Figure 3



445 Figure 4
446



447
448

Figure 1

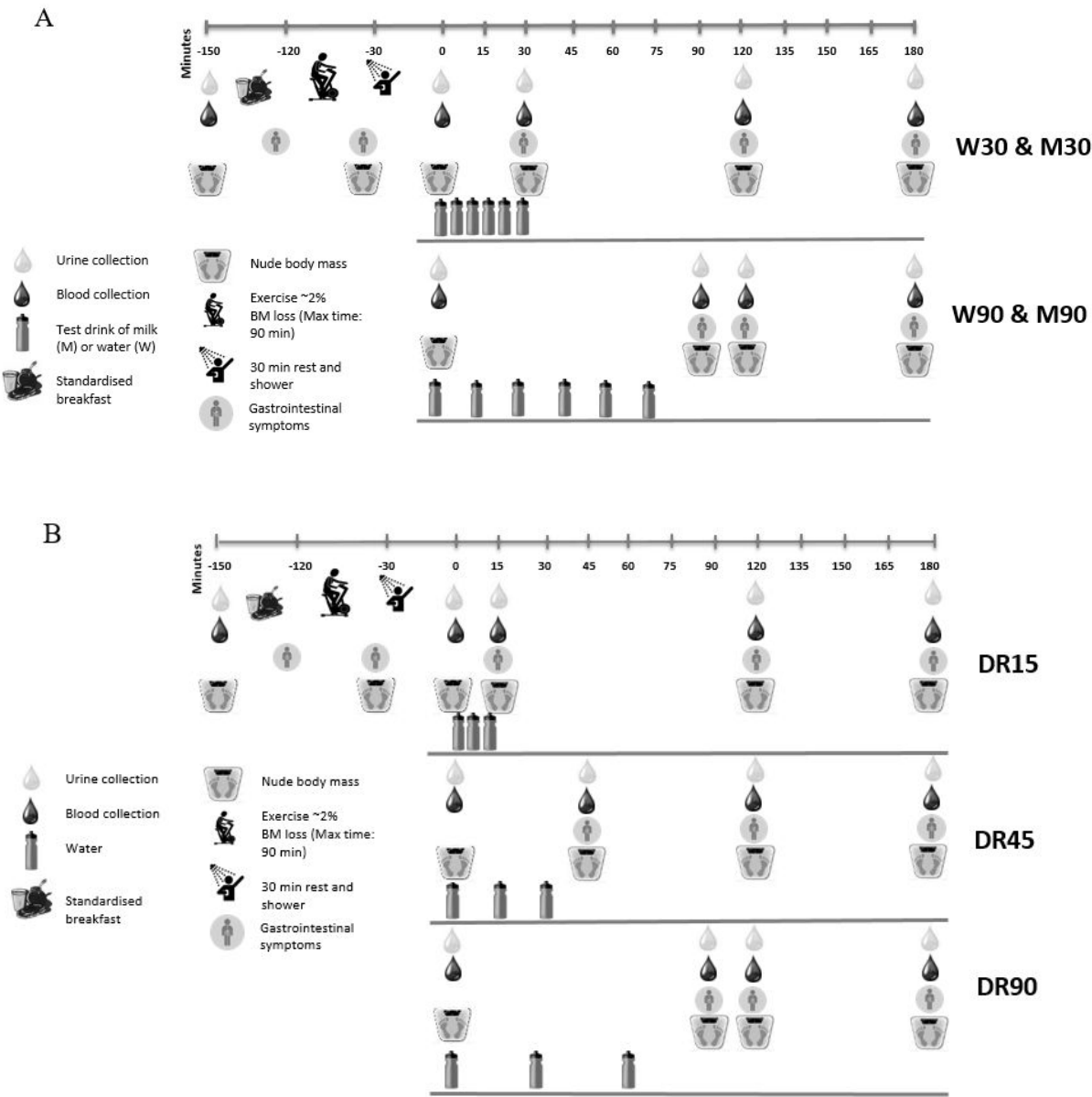


Figure 2

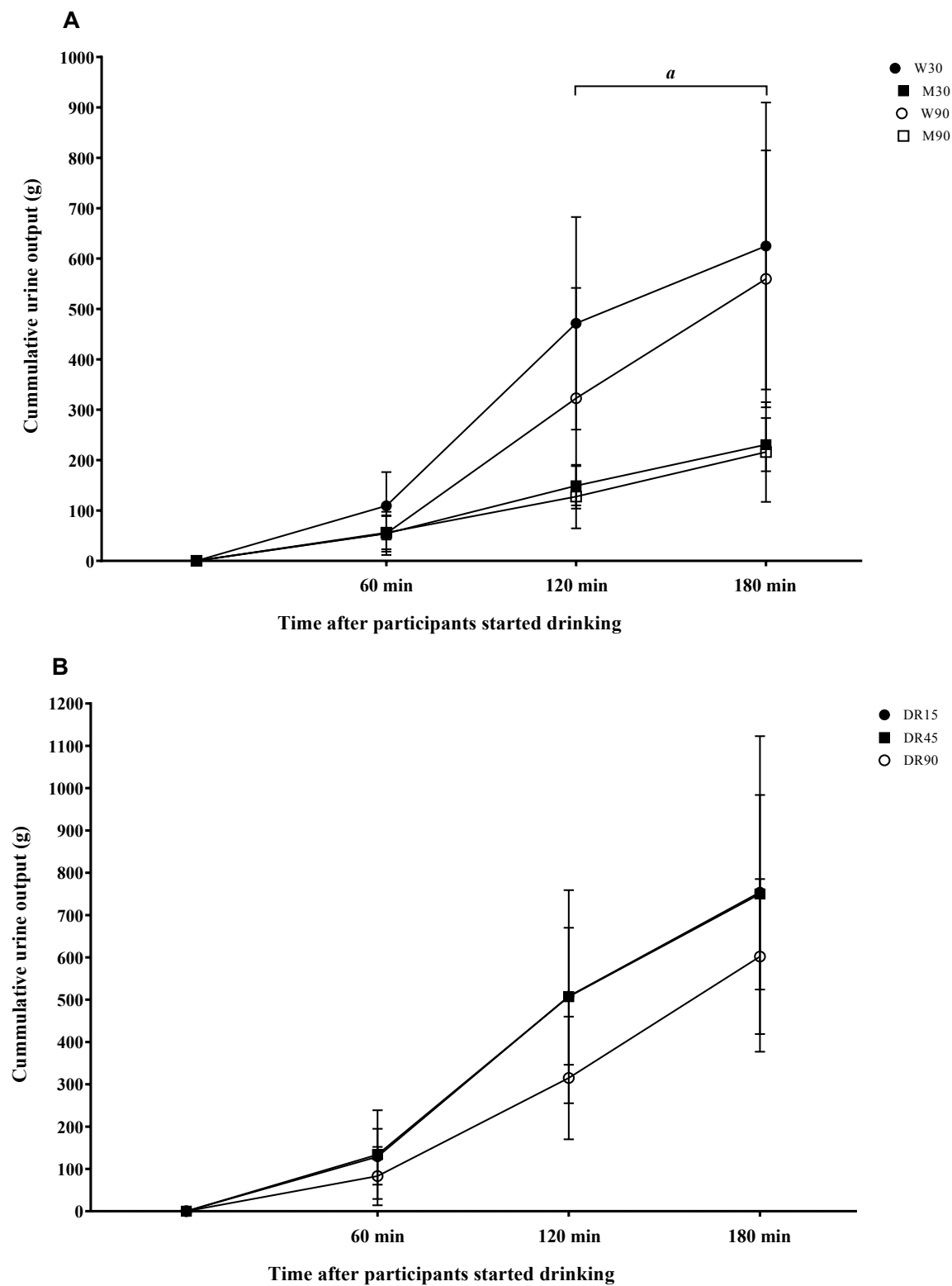


Figure 3

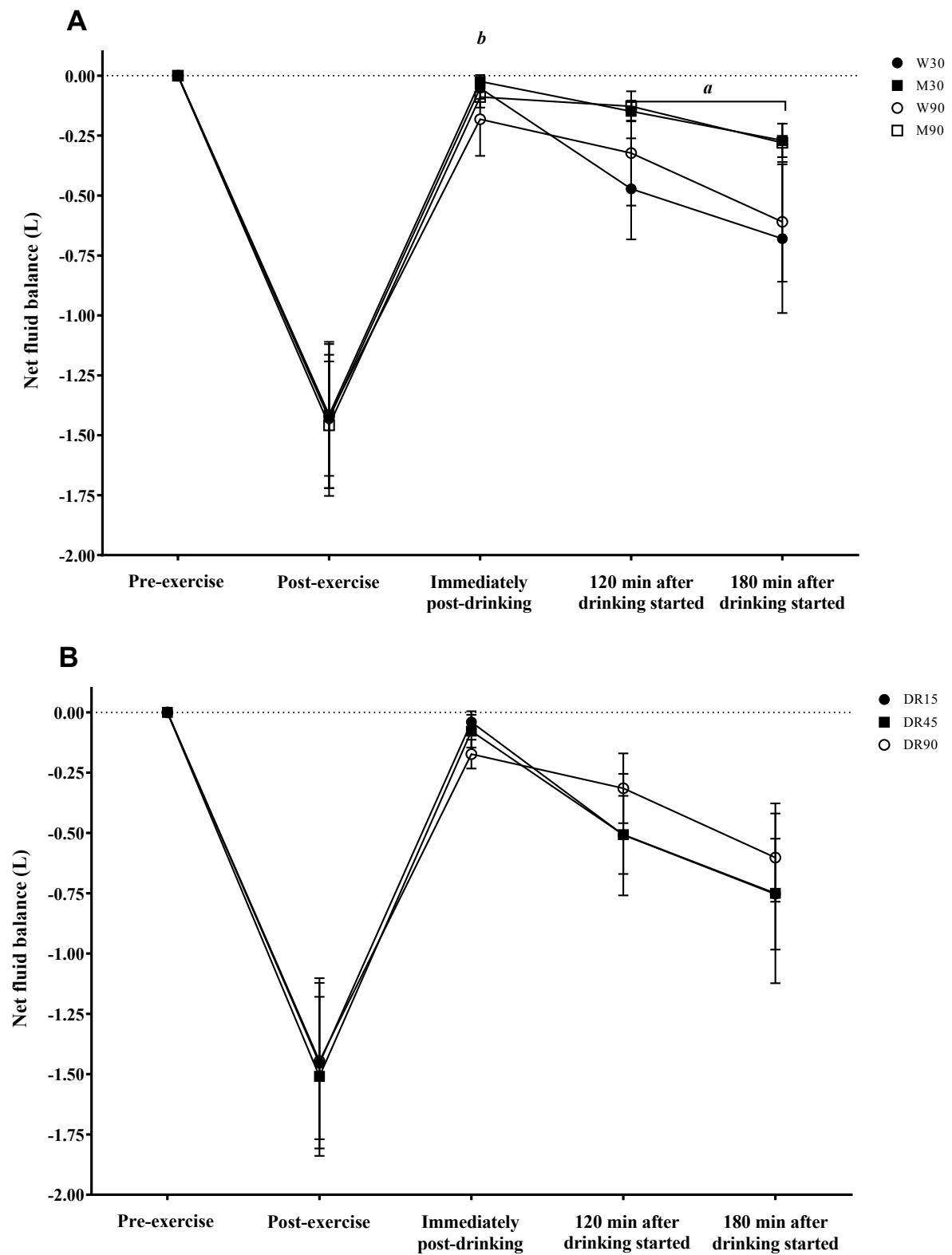


Figure 4

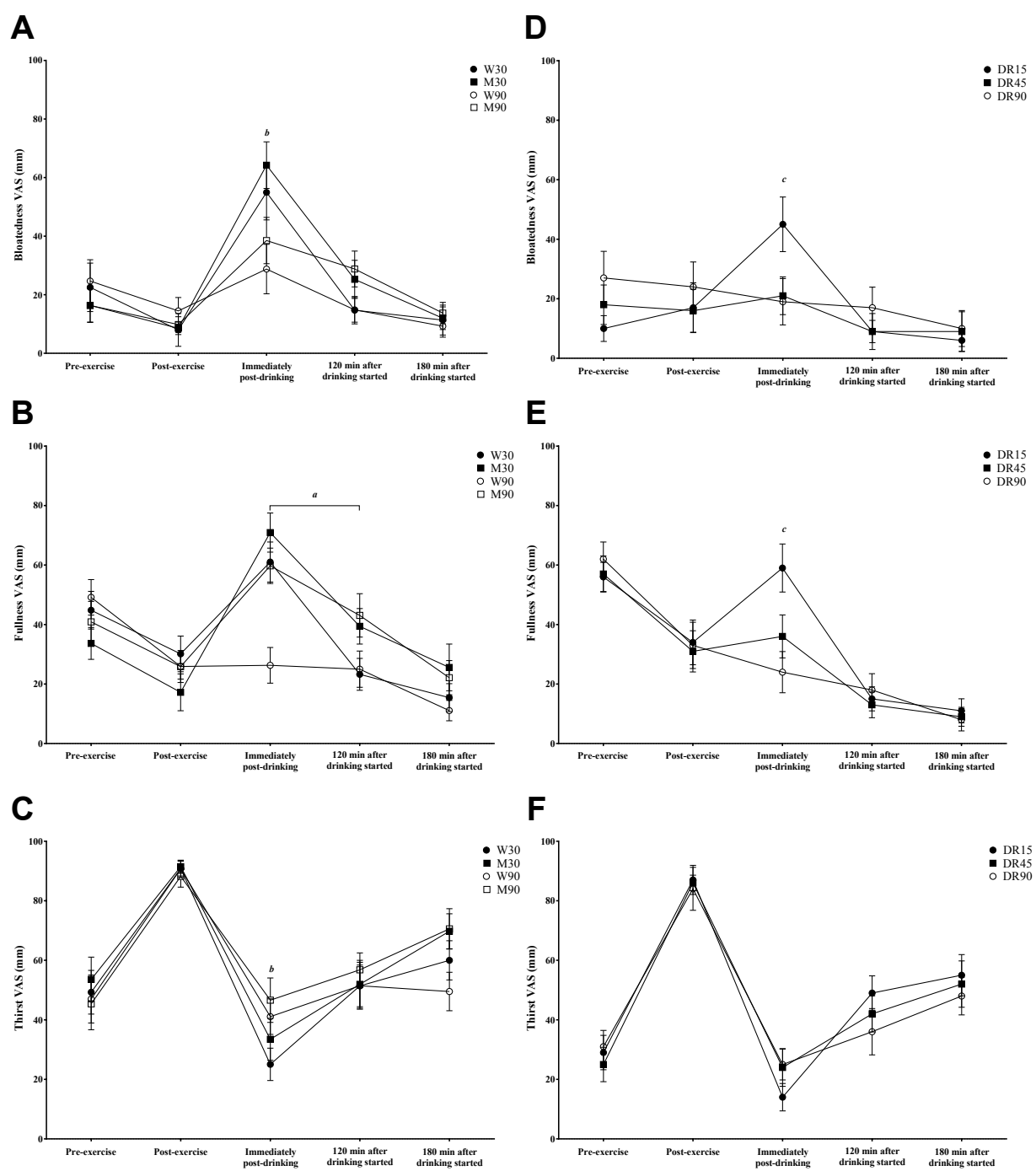


Table 1. Pre-trial conditions and impact of exercise-induced dehydration

| Part A | W30 | W90 | M30 | M90 | <i>p</i>-value |
|---|-------------|--------------|--------------|-------------|-----------------------|
| Pre-Ex U_{SG} | 1.015±0.006 | 1.015±0.007 | 1.013±0.005 | 1.014±0.005 | 0.35 |
| Pre-Ex P_{OSM} (mOsm·kg ⁻¹) | 290±4 | 292±5 | 290±6 | 289±5 | 0.67 |
| Pre-Ex BM (kg) | 77.10±9.67 | 77.27±9.78 | 76.77±9.73 | 76.57±9.52 | 0.28 |
| Ex Duration (min) | 70±14 | 70±13 | 70±13 | 70±12 | 0.86 |
| BM Loss (kg) | 1.46±0.28 | 1.42±0.30 | 1.43±0.32 | 1.46±0.29 | 0.79 |
| BM Loss (%) | 1.9±0.3 | 1.9±0.4 | 1.9±0.4 | 1.9±0.3 | 0.82 |
| Part B | DR15 | DR45 | DR90 | | <i>p</i>-value |
| Pre-Ex U_{OSM} | 477±218 | 474±178 | 443±185 | | 0.76 |
| Pre-Ex P_{OSM} (mOsm·kg ⁻¹) | 303±5 | 302±3 | 302± 5 | | 0.36 |
| Pre-Ex BM (kg) | 71.60±9.90 | 71.54± 10.15 | 71.31± 10.08 | | 0.39 |
| Ex Duration (min) | 79±12 | 81±13 | 80± 11 | | 0.62 |
| BM Loss (kg) | 1.46±0.35 | 1.51± 0.33 | 1.45±0.32 | | 0.30 |
| BM Loss (%) | 2.0± 0.4 | 2.1±0.2 | 2.0±0.3 | | 0.61 |

BM: Body mass; Ex: Exercise; P_{OSM} : Plasma osmolality; U_{SG} : Urine specific gravity; U_{OSM} : Urine osmolality. Values are Mean±SD.

Table 2. Test-retest trial data (Part B: pooled from DR15 and DR45)

| | Initial Trial | Repeat Trial | <i>p-value</i> |
|--|---------------|--------------|----------------|
| Pre-Trial Conditions | | | |
| Pre-Ex U _{OSM} | 483±197 | 479±197 | 0.30 |
| Pre-Ex P _{OSM} (mOsm·kg ⁻¹) | 307±5 | 307±7 | 0.81 |
| Pre-Ex BM (kg) | 72.56±11.10 | 72.38±10.94 | 0.30 |
| Ex Duration (min) | 80.0±13.5 | 80.8±13.1 | 0.34 |
| BM Loss (kg) | 1.43±0.32 | 1.39±0.39 | 0.62 |
| Fluid Retention Data | | | |
| Cumulative urine output (g) | 792± 280 | 704±175 | 0.07 |
| U _{OSM} 180 min after drinking started (mOsm·kg ⁻¹) | 297±75 | 281±127 | 0.69 |
| P _{OSM} 180 min after drinking started (mOsm·kg ⁻¹) | 303±4 | 302±4 | 0.38 |
| Fluid retention (%) | 52.8±7.0 | 55.0±7.5 | 0.21 |
| Values are mean±SD. | | | |